

(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau



(43) International Publication Date
9 August 2001 (09.08.2001)

PCT

(10) International Publication Number
WO 01/057256 A3

(51) International Patent Classification: C12Q 1/68

(21) International Application Number: PCT/US01/03439

(22) International Filing Date: 2 February 2001 (02.02.2001)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
09/498,585 4 February 2000 (04.02.2000) US

(71) Applicant (for all designated States except US): MOLECULAR DYNAMICS, INC. [US/US]; 928 East Arques Avenue, Sunnyvale, CA 94086 (US).

(72) Inventor; and

(75) Inventor/Applicant (for US only): MAHTANI, Melaine, M. [CA/US]; 192 Everett Avenue, Palo Alto, CA 94301 (US).

(74) Agent: RONNING, Royal, N., Jr.; Amersham Pharmacia Biotech, Inc., 800 Centennial Avenue, Piscataway, NJ 08855 (US).

(81) Designated States (national): AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CR, CU, CZ, DE, DK, DM, DZ, EE, ES, FI, GB, GD, GE, GL, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW.

(84) Designated States (regional): ARIPO patent (GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).

Published:

with international search report
— before the expiration of the time limit for amending the claims and to be republished in the event of receipt of amendments

(88) Date of publication of the international search report:
25 July 2002

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

(54) Title: ROLLING CIRCLE AMPLIFICATION ASSAY FOR NUCLEIC ACID ANALYSIS

(57) Abstract: Method and reagents for analysis of nucleic acid sequences is disclosed. In this method a plurality of padlock probes is provided. Each padlock probe may hybridize to a locus on a target nucleic acid under hybridization conditions. If a targeted variant is present at the locus, the padlock probe may be ligated to form an amplification target circle. The amplification target circle acts as a template for production of tandem-sequence DNA. The tandem-sequence DNA may then be digested into non-tandem detection fragments which are subsequently separated and detected. The plurality of padlock probes are designed such that ligation of the probes, amplification of the target circle, and digestion of the tandem-sequence DNA subsequently produced, and detection may all be effected with the same set of reagents. Each probe targets a unique locus variant on the target nucleic acid sequence and produces a detection fragment that may be distinguished from detection fragments produced from other padlock probe in the plurality of padlock probes by using a fragment analysis detector.

WO 01/057256 A3

INTERNATIONAL SEARCH REPORT

Int. Application No.

PCT/US 01/03439

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 C12Q1/68

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 7 C12Q

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the International search (name of data base and, where practical, search terms used)

EPO-Internal, MEDLINE, BIOSIS, WPI Data, PAJ

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	LIZARDI P M ET AL: "MUTATION DETECTION AND SINGLE-MOLECULE COUNTING USING ISOTHERMAL ROLLING-CIRCLE AMPLIFICATION" NATURE GENETICS, NEW YORK, NY, US, vol. 19, no. 3, July 1998 (1998-07), pages 225-232, XP000856939 ISSN: 1061-4036 the whole document	1-11
A	WO 00 04193 A (UNIV YALE) 27 January 2000 (2000-01-27) page 15 page 24-25 page 53-58; claims	1-28
A	US 5 854 033 A (LIZARDI PAUL M) 29 December 1998 (1998-12-29) column 1-3; claims; example 5	1-28
-/-		

☒ Further documents are listed in the continuation of box C.☒ Patent family members are listed in annex.*** Special categories of cited documents:**

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"Z" document member of the same patent family

Date of the actual completion of the international search

8 May 2002

Date of mailing of the international search report

17/05/2002

Name and mailing address of the ISA
European Patent Office, P.B. 5618 Patentlaan 2
NL - 2260 HV Rijswijk
Tel. (+31-70) 340-2040, Tx. 31 651 epo nl,
Fax: (+31-70) 340-3016

Authorized officer

Reuter, U

INTERNATIONAL SEARCH REPORT

Int. Application No.
PCT/US 01/03439

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	WO 99 09216 A (UNIV ROCHESTER) 25 February 1999 (1999-02-25) page 6-9; figures 1,2 page 34-35 -----	1-28

INTERNATIONAL SEARCH REPORT

 Int. Application No
 PCT/US 01/03439

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 0004193 A	27-01-2000	EP 1098996 A1	16-05-2001
		WO 0004193 A1	27-01-2000
		US 6316229 B1	13-11-2001
US 5854033 A	29-12-1998	AT 199572 T	15-03-2001
		AU 714486 B2	06-01-2000
		AU 1024097 A	11-06-1997
		DE 69612013 D1	12-04-2001
		DE 69612013 T2	02-08-2001
		DK 862656 T3	09-04-2001
		EP 0862656 A1	09-09-1998
		JP 2002503948 T	05-02-2002
		WO 9719193 A2	29-05-1997
		US 6183960 B1	06-02-2001
		US 6210884 B1	03-04-2001
		US 6329150 B1	11-12-2001
		US 6344329 B1	05-02-2002
		US 6143495 A	07-11-2000
WO 9909216 A	25-02-1999	US 6077668 A	20-06-2000
		AU 8782198 A	08-03-1999
		EP 1019535 A2	19-07-2000
		WO 9909216 A2	25-02-1999